Issues to be Addressed in Platelet Radiolabeling

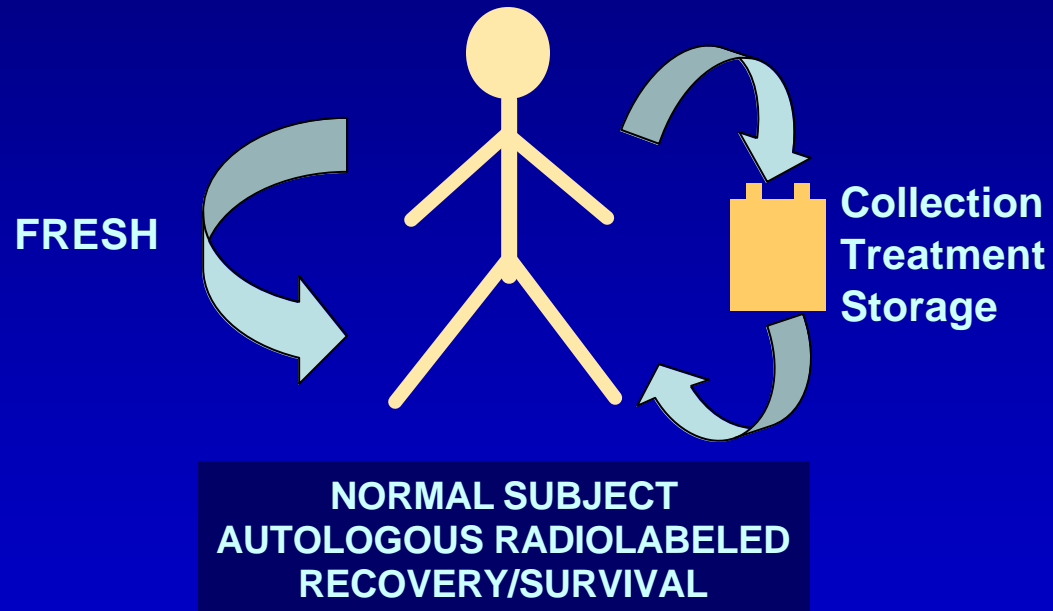**James P. AuBuchon, MD**
Dartmouth-Hitchcock Medical Center
Lebanon, New Hampshire

Issues to be Addressed in Platelet Radiolabeling

The changes in new models should be so attractive as to create dissatisfaction with past models.

- Sloan's Law

The Concept

FRESH

Collection
Treatment
Storage

**NORMAL SUBJECT
AUTOLOGOUS RADIOLABELED
RECOVERY/SURVIVAL**
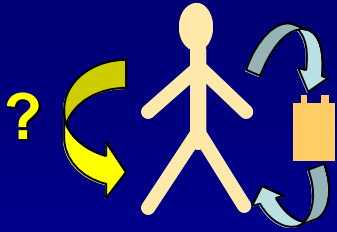
The Details

Source of Comparative Standard
Source of Fresh Platelets
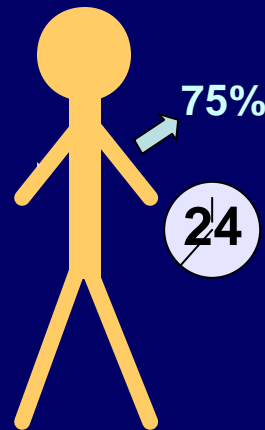**Method of Collection of Fresh Platelets**
Timing of Reinfusion of Fresh Platelets
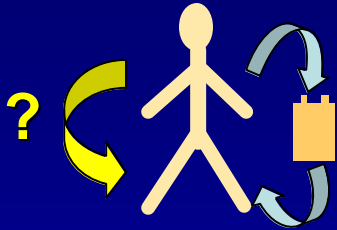**Technical Details of Radiolabeling Protocol**

Source of Comparative Standard

Does the standard need to come from the same subject?
Could the comparison be against an absolute criterion?

?

75%

24

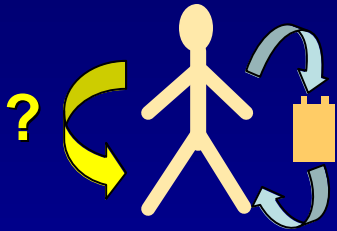# Source of Comparative Standard

Does the standard need to come from the same subject?
Could the comparison be against an absolute criterion?

Using an absolute standard

+ Mirrors approach taken with Red Blood Cells
+ Could be followed by "fresh" study if results poor

Source of Comparative Standard

?

Does the standard need to come from the same subject?
Could the comparison be against an absolute criterion?

<u>Using the subject as his/her own control</u>
+ Accommodates subject individualities
+ Allows compensation for lab variability
+ Accounts for "drift" in procedures/results over time
+ May limit number of observations/exposures needed (pairing)
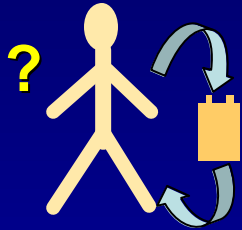
Source of Fresh Platelets
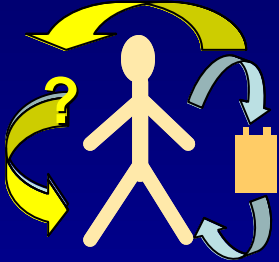
Should the fresh platelets be an aliquot of the unit taken from it shortly after collection or collected separately?

<u>Using a separate collection</u>
+ Prevents damage at collection from creating a lower standard
+ Avoids variability by collection technique

Source of Fresh Platelets

? *Should the fresh platelets be an aliquot of the unit taken from it shortly after collection or collected separately?*

Using an aliquot from the unit
- **+ Accurate representation of the population in unit**
- **+ Apheresis techniques may be less injurious**
- **+ Inexpensive, simple**

Collection of Fresh Platelets
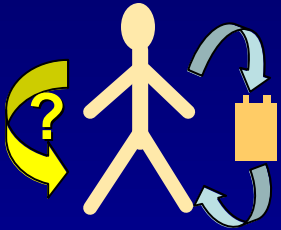
If the "fresh" platelets are not an aliquot of the unit, how should they be collected?

<u>Collection of a whole blood unit</u>
+ Allows application of considerable experience in unit preparation

Collection of Fresh Platelets
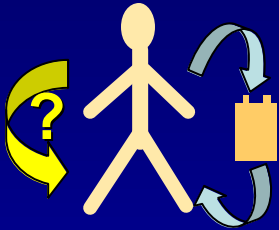
If the "fresh" platelets are not an aliquot of the unit, how should they be collected?

Small volume collection/harvest

+ Allows standardization of entire process
+ Least disruptive of subject's blood volume
+ Red cell reinfusion avoided

Collection of Fresh Platelets
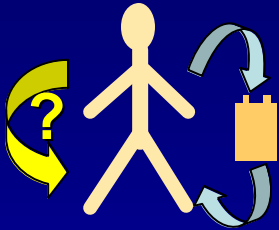
If the "fresh" platelets are not an aliquot of the unit, how should they be collected?

Collection by apheresis
   + Some instruments cause little platelet damage

Timing of Reinfusion of Fresh Platelets
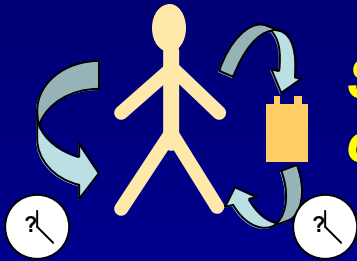
Should fresh platelets be reinfused on Day 0/1 or on the day of reinfusion of the stored platelets?

<u>Reinfusion on day of collection</u>

+ Allows use of platelets that reflect those being stored

+ If storage period long enough, use same label for both arms

+ Good intra-subject reproducibility reported over time

Timing of Reinfusion of Fresh Platelets

Should fresh platelets be reinfused on Day 0/1 or on the day of reinfusion of the stored platelets?
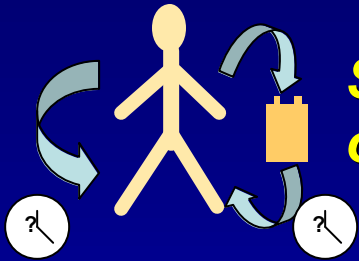
Reinfusion on same day as test platelets
   + Reduces variability in subject status at time of reinfusion

Timing of Reinfusion of Test Platelets
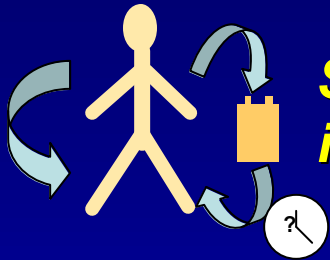
Should test platelets be reinfused on the last day of intended storage or the following day?

Reinfusion on last day of storage
    + Current method
    + Would allow comparison with previous studies

Reinfusion on day after last day
    + Provides assurance of functionality to/beyond intended outdate

Technical Details of Platelet Radiolabeling
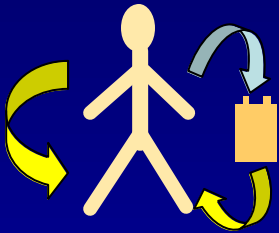
Radioactivity dosage
- What radioactive dose should be injected?
- Should a minimum for counts acquired be required?
- Should high efficiency (3-inch crystal) counters be required?
- Should some threshold over background be required?

Platelet content
- Should the specified dose be distributed among a defined number of platelets?

Technical Details of Platelet Radiolabeling
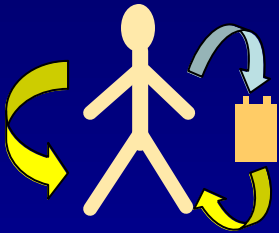
Labeling environment
In what should platelets be suspended?
- ACD-A/saline
- Ringer's-Citrate-Dextrose

Labeling vessel
In what container should the labeling occur?
- Plastic bag
- Conical tube

Technical Details of Platelet Radiolabeling
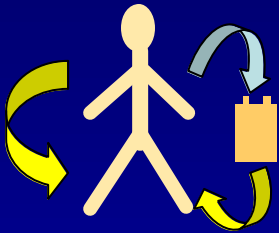
Sampling times
5 min, 1 h, 3 h and "daily for 10 days"
- Is accommodation of weekends with two samples on Friday and two on Monday acceptable?
- Are all these samples necessary? What is the minimum number of samples acceptable for determining the survival curve
  – over how many days?

How should recovery be determined?
- Highest of the first three points
- The 1 h or 3 h sample
- Back-extrapolation from the survival curve

Technical Details of Platelet Radiolabeling
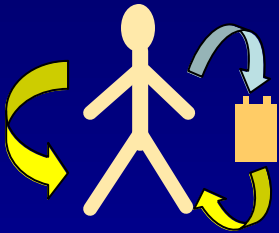
How should the subject's blood volume be estimated?
- Indirectly, based on height, weight and gender (which formula?)
- Directly, through radiolabeled autologous red cells

How should the recovery curve be calculated and stated?
- As the numerical expected lifespan
- Using which mathematical model (multiple hit?)
- Using which computer software (COST?)

Technical Details of Platelet Radiolabeling
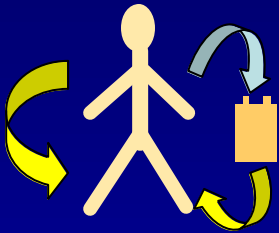
Is there any value to calculation of the area under the curve?

- Excessive reliance on survival parameter?
- Calculate as linear approximation (recovery * survival * 0.5)?
- Determine via COST program?

What corrections should be included in the calculations?

- Correction for elution of the label prior to the injection
- Correction for radioactivity in the plasma of a sample
- Correction for red cell labeling

Sample Protocols

FRESH

Collection
Treatment
Storage

**NORMAL SUBJECT
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RECOVERY/SURVIVAL**
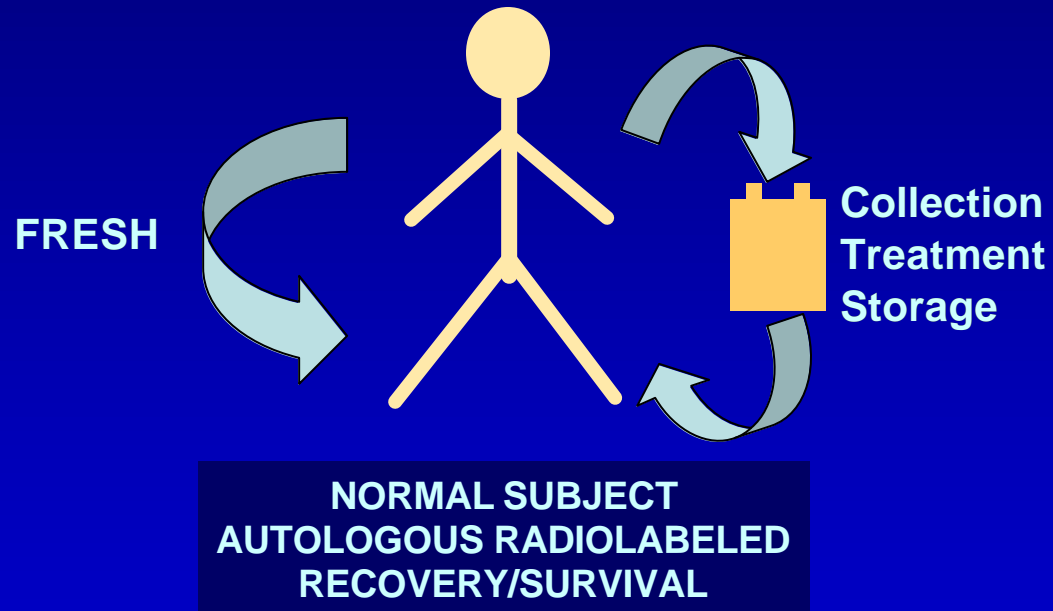
Comparison Against Absolute Standard

```
┌─────────────────────────┐          ┌─────────────────────────────────────┐
│ Lab validates procedure │          │   Determination of Standard         │
│ as giving expected result│         │ 70% recovery (max) * 67% = 47%      │
└─────────────────────────┘          │ 9 d survival (max) * 50% = 4.5 d    │
            │                        └─────────────────────────────────────┘
            ▼                                          │
┌─────────────────────────┐                           │
│ Lab repeats validation  │                           │
│ periodically            │                           ▼
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐        ┌─────────────────────────┐
│ Clinical trial conducted│───────▶│ Accept/reject method    │
└─────────────────────────┘        └─────────────────────────┘
            │                                   ▲
            ▼                                   │
┌─────────────────────────┐                     │
│ Unexpected results      │                     │
│ encountered             │                     │
└─────────────────────────┘                     │
            │                                   │
            ▼                                   │
┌─────────────────────────┐                     │
│ Study results with fresh│                     │
│ platelets from these or │                     │
│ all enrolled subjects   │                     │
└─────────────────────────┘                     │
            │                                   │
            ▼                                   │
┌─────────────────────────┐                     │
│ If not explained, enroll│─────────────────────┘
│ additional subjects (?) │
└─────────────────────────┘
```
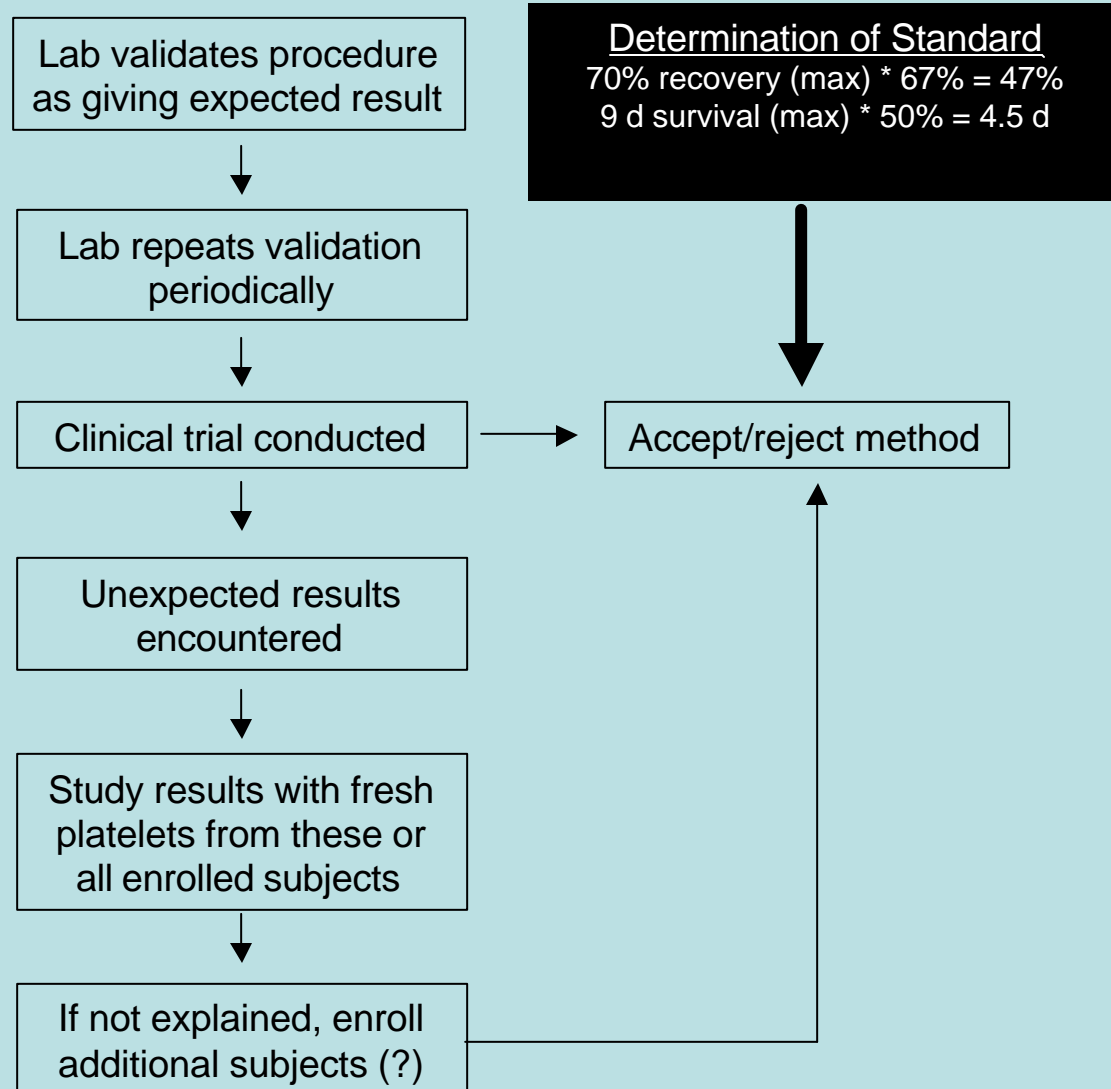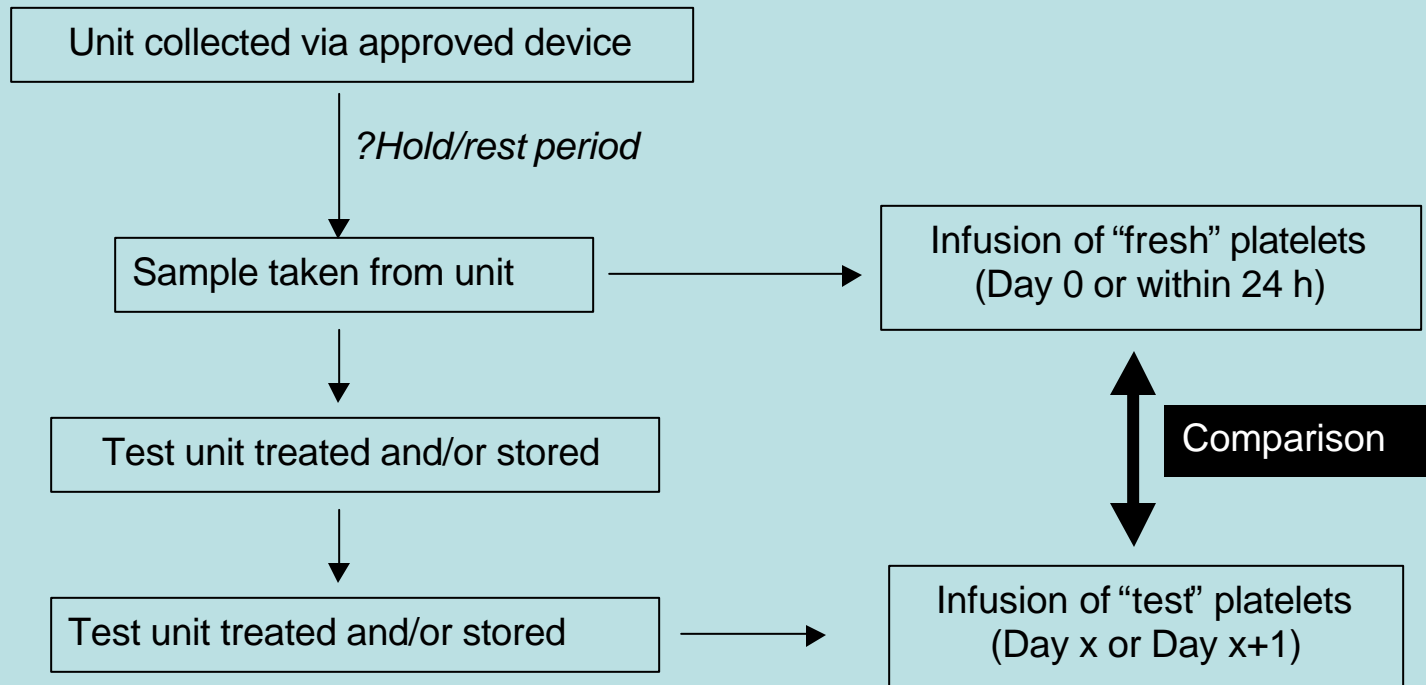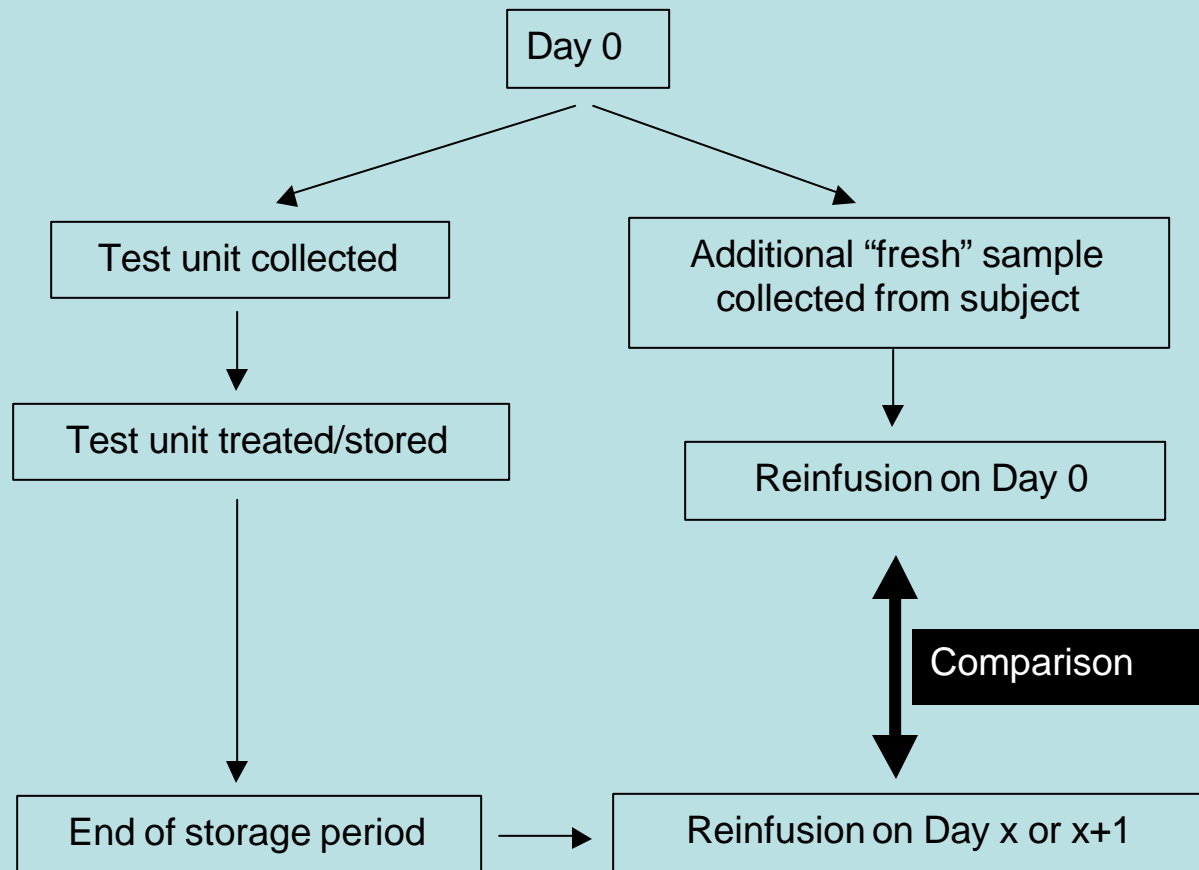
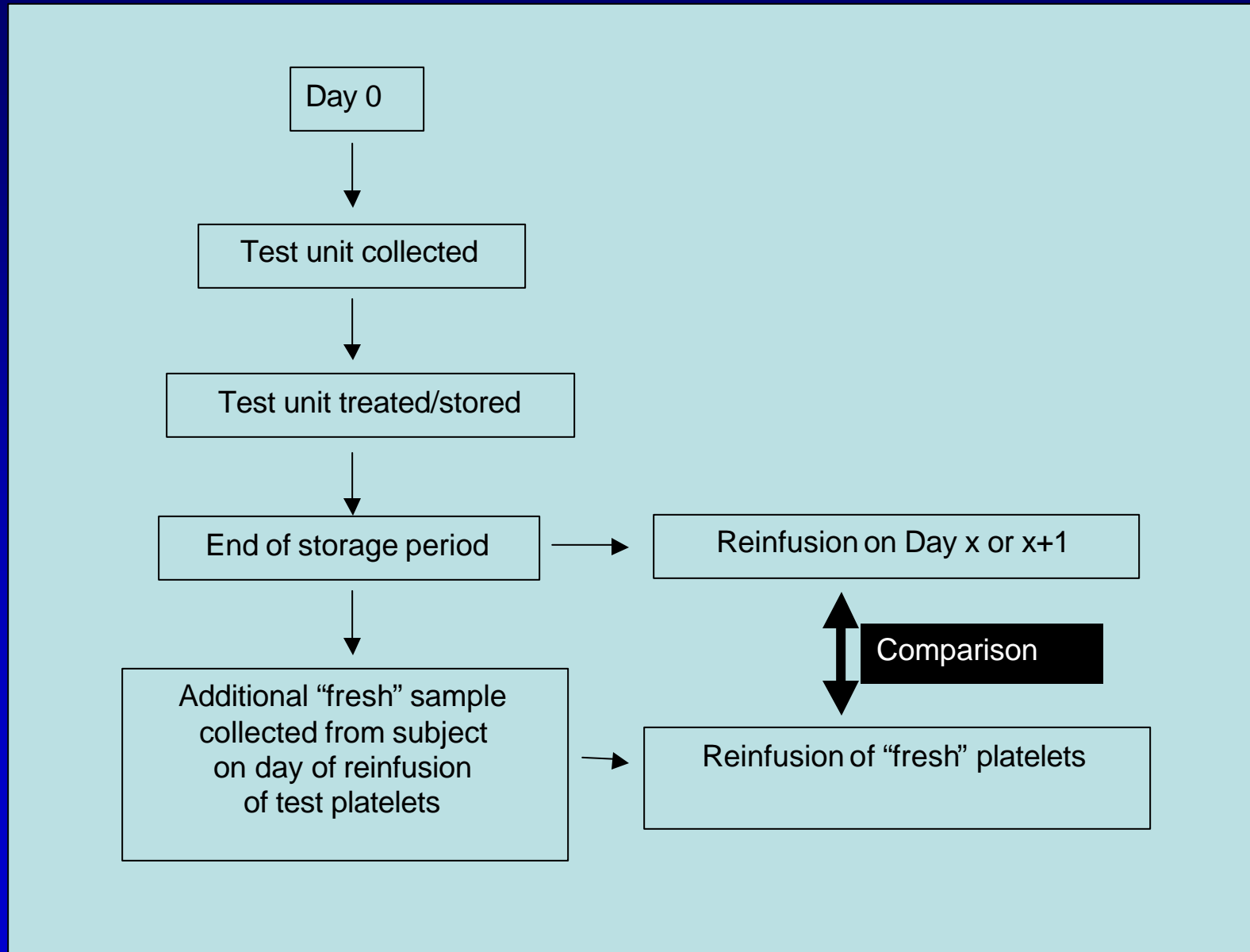# Comparative Standard Using Sample from Unit
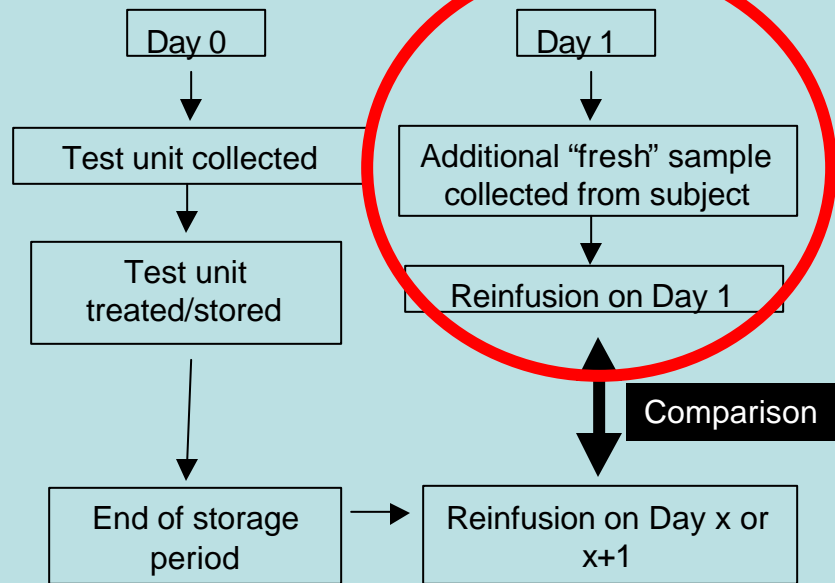
Comparative Standard Using Sample on Day of Collection
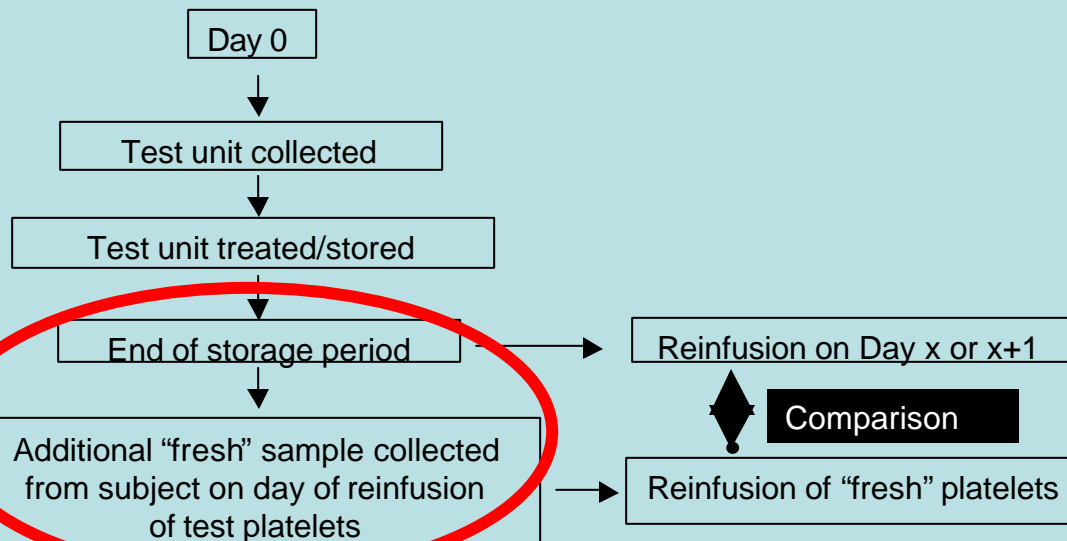
Comparative Standard Using Sample on Day of Reinfusion

Comparative Standard: Different Approaches

Test: Apheresis platelets

Test: Whole blood-derived platelets